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The role of carbohydrate in the function of human plasminogen: comparison of the protein obtained from molecular cloning and expression in *Escherichia coli* and COS cells

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A cDNA library was constructed in the phage lambda gt11 from human liver mRNA enriched for plasminogen mRNA by chromatography on Sepharose 4B. A full-length cDNA clone of human plasminogen was isolated. The 2.7 kb cDNA encoded the entire plasminogen molecule, a signal peptide sequence and two start codons with a 5′-untranslated region of about 80 base pairs. In the 3′-non coding region of 280 base pairs a consensus signal AATAAA was found at a distance of 46 base pairs upstream of the poly(A) tail. The plasminogen cDNA was subcloned in the eukaryotic expression vector p91023 (B), and human plasminogen was expressed in monkey kidney (COS m6) cells and in *Escherichia coli*. The recombinant molecule obtained from COS cells has physicochemical and biological properties similar to native human plasminogen I, indicating that it has folded in a manner similar to plasminogen synthesized by liver. By contrast, plasminogen expressed in *E. coli* could not be activated and showed biological properties which are very different from glycosylated forms of plasminogen. However, the non-glycosylated plasminogen was bound by lysine-Sepharose and reacted with a conformation dependent monoclonal antibody to kringles 1 to 3. These data suggest that the protein has properly folded kringle domains. Our studies suggest that the carbohydrate domains may play an important role in the function of the plasminogen molecule.

Introduction

The glycoprotein plasminogen is a zymogen that participates in the final stages of fibrinolysis [1]. The one-chain zymogen is activated by a number of activators, including tissue plasminogen activator, urokinase or a complex of plasminogen and streptokinase. The active two chain molecule, plasmin, results from cleavage of the peptide bond between Arg-560 and Val-561 [2]. Two major forms of plasminogen have been separated on L-lysine-Sepharose [3]. Form I contains two carbohydrate chains linked to Asn-280 and Thr-345, while form II contains one carbohydrate chain linked to Thr-345 [4,5]. The activation of plasminogen I is enhanced more than that of plasminogen II in the presence of fibrin by either urokinase or streptokinase [6]. Plasminogen is synthesized in the liver [7–9]. The primary structure of plasminogen has been determined in mixtures containing both forms [10,11], but a similar primary structure for isolated plasminogen I and II has not been rigorously proven by protein sequence analysis. It is known that the synthesis of the two forms in monkey liver is directed by 23 and 18 S mRNAs [12].

A partial cDNA sequence for the plasminogen gene has been published [13]. Recently, a full-length plasminogen cDNA has been reported [14], and the expression of human plasminogen in a baculovirus vector-infected cell system has been achieved [15]. In the present study, we report the isolation of a full-length cDNA for plasminogen and the expression of human plasminogen in *Escherichia coli* and monkey kidney (COS m6) cells, with the novel observation that the carbohydrate domains play an important physiological role in the function of the plasminogen molecule both with respect to activation and endothelial recognition.

Abbreviations: IPTG, isopropyl-D-thiogalactopyranoside; t-PA, tissue plasminogen activator; DFP, diisopropyl fluorophosphate.

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